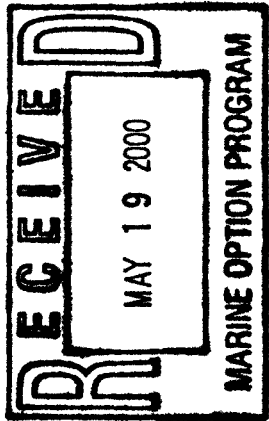


Survey of toxic dinoflagellate populations on the big island of Hawai'i

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Abstract



Macrophyte samples were collected at three sites around the Big Island of Hawai'i over a 5-month period. Sampling sites included Mahai'ula beach park (leeward side), Richardson's beach park (windward side) and Onekahakaha beach park (windward side). To study the distribution of epiphytic ciguatoxic dinoflagellates, samples were stained with calcofluor and examined with an epifluorescence microscope equipped with a DAPI filter. Ciguatoxic dinoflagellates were identified and enumerated. Results demonstrated that Mahai'ula's (leeward side) had high counts of *Gambierdiscus toxicus*, while Richardson's had high counts of *Prorocentrum* spp. on *Galaxaura marginata* and *Trichleocarpa fragilis*. *Ostreopsis ovata* was found in higher numbers in the fall and sites within fall. Dinoflagellate abundance was compared with location, season, macroalgal biomass and species, and water conditions. These findings support the hypothesis that ciguatera is found on the leeward side of Hawaiian Islands, but makes us look at the windward side also because of the large count of toxic dinoflagellates.

Ciguatera symptoms were first described in the Caribbean in the late 1500's, when Spanish explorers linked the symptoms to the ingestion of a snail that they termed *cigua* (Blythe 1999). In 1774 another historical poisoning event happened in the tropical

Pacific when Captain Cook and his crew became very sick after eating red snapper (Banner 1965). Since these early descriptions of ciguatera symptoms, poisoning events have been occurring at an ever-increasing rate, causing the public and scientific communities to be aware of the pending health and fisheries hazard. Presently, as the world's population continues to grow at a rate faster than ever before, humans are being forced to rely on the ocean's resources more in their daily activities (Fleming 1998). As a result, food poisoning incidents involving aquatic toxins are beginning to spread to the outer reaches of most continental regions.

Ciguatera is endemic to the latitudes of 34 degrees south and 35 degrees north, and is the leading cause of food poisoning in circumtropical areas affecting over 50,000 people annually (Legrand 1998). This number may be significantly underestimated due to the increase of international seafood exportation and the lack of clinical diagnostics when determining ciguateric symptoms (Fleming 1998). While endemic to these areas ciguatera outbreaks in Hawai'i have been reported in much higher cases on the leeward side (west coast) relative to the windward side (east coast) by both scientist's and fishermen around the island's. Studies have been conducted along the coasts of Hawai'i, but no significant data has ever been published on the subject.

Ciguatera fish poisoning (CFP) is caused by a group of benthic and epiphytic dinoflagellates (division Pyrrophyta) that live on macro-algae, coral, or sandy substrates. *Gambierdiscus toxicus* Adachi et Fukuyo was the first dinoflagellate to be classified in the ciguatera category in 1979 and was thought to be the primary toxic species involved in CFP (Chinain 1999a). Since 1979, the two toxins produced by *Gambierdiscus toxicus*, ciguatoxin (CTX) and maitotoxin (MTX), have been combined with palytoxin

(PTX), and brevetoxin (PbTx), which are produced by other dinoflagellates. This group of toxins has been placed in the “ciguatera category” because of their similar effects and mutual occurrence in fish species (Morton 1997). Other dinoflagellates that have been identified in the ciguatera category include *Prorocentrum* spp., *Ostreopsis* spp., *Amphidinium klepsii*, and *Coolia monotis*, although the role they play in the ciguatera epidemic is unclear at this time (Legrand 1998). Recent studies have also found that okadaic acid, produced by *Prorocentrum* spp., to be a potent tumor promoter on mouse skin and glandular stomach (Huynh 1998). In another paper, Landsberg et al. (1999) hypothesized that the okadaic acid is a causative factor in the formation of fibropapillomatosis in green sea turtles living in Hawaiian and other coastal waters.

The toxins in the ciguatera category are very strong neurotoxins. The extent of the effects on the human body are determined by the amount of toxin that is ingested. The toxin is derived from food chains that revolve around the ingestion or scraping of the substratum that harbor the toxic dinoflagellates. Common reef fish including groupers, snappers, barracuda, mackerels and jacks usually incorporate the largest amount of ciguatoxin due to their order and role in the reef ecosystem (Steidinger 1993).

Ciguatera symptoms include weakness, abdominal cramps, diarrhea, nausea, numbness of digits, temperature-reversal, muscle ache, dizziness, itching, sweating, paralysis, and may even result in death (Delgado 1996). Once the ciguatoxin enters the body it is absorbed by fatty tissues. Symptoms can last for days, months, or even years and have been known to reappear when coupled with alcohol, heat, and repeated ingestion of infected fish.

Currently, there is not a cure for ciguatera, but there are medicines that can reduce the effects of certain symptoms. The only preventative measure at the present time is to test fish before they are eaten. There are several different ways to test for toxicity, including an on-site test kit, but improving the validity and efficiency of these is very important in order to reduce food poison reports.

One research focus is on the dinoflagellate life cycle, which may provide insight into the current proliferation of poisoning reports. Dinoflagellates obtain their nourishment through the process known as photosynthesis, which can be limited by several factors in the ocean. If these factors are no longer limiting, a bloom can occur and introduce a large amount of toxin into the food chain. Resorts, golf courses and other anthropogenic sources are notorious for contributing to substrate disturbance and fertilizer input of coastal communities. It has been hypothesized that these factors plus global climate change are a catalyst in both bloom growth and poisoning events (Fleming 1998).

In order to gain a better understanding of the dinoflagellates that cause ciguatera, one must begin to study the organism from a basic level. In my research project, I studied several different sites around the big island of Hawaii to determine what areas that toxic dinoflagellates are endemic to. By understanding where the dinoflagellates are most commonly found, we can begin to study these sites exclusively to discover why the dinoflagellates thrive in these areas. As we begin to gain an understanding of the organism's ecology, we can attempt to control the factors that lead to an increase in ciguatera poisoning. To evaluate the relevance of site specific ciguatoxic poisonings studied different sites around the big island of Hawai'i by collecting macrophyte samples

that will be examined in the laboratory at a later time. My hypothesis is that there will be a greater occurrence of toxic dinoflagellates on the leeward side of the big island relative to the windward side. My second hypothesis is that there will be a difference in the population densities of dinoflagellates on different algal species.

H₁: there will be a difference in the population density of toxic
dinoflagellates between sites

H₂: there will be a difference in the population density of toxic
dinoflagellates between algal species

Materials and Methods

Data collection took place over an elapsed period of six months, starting in October of 1999 and ending in February of 2000. The sampling was separated into a fall group (late October-early November), and a winter group (January). Three sites (Fig. 1) were chosen around the island of Hawai'i with the intention of getting an east-west correlation between sites: Mahai'ula beach located on the leeward coast, 80 kilometers North of Kailua; Richardson's beach park located on the windward coast, 13 kilometers south of Hilo; and Onekahakaha beach park located on the windward coast, 5 kilometers south of Hilo. Sampling methods were carried out by snorkeling since water depth at all sampling areas did not exceed 3 meters. Macrophyte specimens were collected randomly at each site by sectioning the macrophyte directly into container with their abundance at the time of sampling. 1-3 samples of each the most common species were collected. Samples were later preserved with 4% formaldehyde solution. Dinoflagellates were dislodged by vigorously shaking the container for one minute.

0.8 ml of solution was removed from the center of the container with a pipette and filtered through a 1.0 μ m polycarbonate filter using a filtering apparatus (Fig. 2) and vacuum pump. Using the method modified from Fritz (1985), 3 drops of 6 mg/l of Calcofluor White M2R (Polysciences, Warrington, PA) were placed on each filter and left to absorb for 10 minutes. Samples were then immediately washed two times with deionized water and mounted on slides with two drops of immersion oil. Calcofluor is a stain that readily bonds to the cellulose of thecal plates in armored dinoflagellates. The stain causes the dinoflagellates to absorb light at a wavelength of 340-400 nm and re-emit visible blue light. A Zeiss epifluorescence microscope equipped with a DAPI filter was used to count and enumerate the dinoflagellates on each side with a top-bottom, left-right scan of the viewing field. Samples were later enumerated and related to the number of toxic dinoflagellates per wet weight of algal matter, per milliliter of total solution of the individual sample, and per milliliter of macrophyte volume displacement. Dinoflagellate species counted included *Gambierdiscus toxicus*, *Ostreopsis lenticularis*, *O. siamensis*, *O. ovata*, and *Prorocentrum* species.

Dinoflagellate counts were quantified back to macrophyte samples using three estimates: wet weight of macrophyte, total solution of sample, and volume displacement of sample. Algal samples was obtained by removing limu from the container, letting drip dry for one minute and weighing. Total solution and volume displacements were measured with a graduated cylinder. The number of dinoflagellates from each sample were then divided by the wet weight, solution, and displacement (# of dinoflagellate/ ml or mg). Counts of toxic dinoflagellates were computed to the number of toxic dinoflagellates per gram of wet weight, per milliliter of total solution of each sample, and

per milliliter of volume displacement of the macrophyte. The results (Fig. 3) of the graphic analysis showed the same trend for the # of dinoflagellates per wet weight, total solution, and volume displacement. Since dinoflagellates use their flagella or a mucous to attach to a surface it was necessary to find a correlation between the dinoflagellates and the macrophytes they were attached to. Volume displacement is the best representative of the surface area that dinoflagellates have to attach themselves, so the number of toxic dinoflagellates per volume displacement of the macrophyte will be used to interpret the data that will be presented. Salinity and temperature at every site were too similar to make any correlation, so this data was ignored.

All macrophyte samples were identified and made into vouchers with the help of Dr. Karla McDermid (University of Hawai'i at Hilo). One replicant from each site was counted three times for both the fall and spring seasons giving a total of 18 replicants.

Results

Spring samples showed an overall decrease of relatively 66% in population density of some toxic dinoflagellates and almost 100% of others. It should be noted that macrophyte species are different than the samples collected during the fall season.

Mahai'ula fall results (Fig. 4) showed *G. toxicus* (1.3/ ml) to have a high population density relative to the other species (.30-.50/ ml) that were present at this site (*O. lenticularis*, *O. ovata*). Mahai'ula's spring samples (Fig.7) showed a similar pattern to the samples from Mahai'ula fall. *G. toxicus* and *O. lenticularis* were the only two species that were present. *O. lenticularis* was the most dense species (2/ ml), with *G.*

toxicus being slightly lower at (1/ ml). Only one species was found on each sample. It should be noted that *Martensia fragilis* was the seaweed at Mahai'ula in the spring.

Richardson's fall samples (Fig. 5) showed an extremely high amount of *Prorocentrum* spp. (70/ ml) with *O. ovata* being present in high abundance (4-12/ ml) relative to other sites. All other toxic dinoflagellates were present in low densities (<2/ ml). *Prorocentrum* spp. were found exclusively on both samples of *Galaxaura marginata* showing a significant preference for this seaweed. *O. ovata* not only had high densities, but appeared on six out of the eight seaweed species, showing a non-exclusive habitat preference. Richardson's spring samples (Fig. 8) show the presence of all toxic dinoflagellates following the fall samples. *Prorocentrum* spp. is the most populous species (20/ ml) and is only present on *Trichleocarpa fragilis*, showing again that *Prorocentrum* spp. is host specific. All of the other toxic dinoflagellate are in very low densities (3/ ml).

Onekahakaha fall samples (Fig. 6) showed low densities of *O. ovata* (.50-2.50/ ml) relative to Richardson's, but showed a similar pattern by being present on seven of the eight species of seaweed collected. This showed once again that *O. ovata* does not prefer a species exclusive environment. Onekahakaha spring samples (Fig. 9) only had two species of dinoflagellates, *Prorocentrum* spp., and *O. lenticularis*. *Prorocentrum* spp. was the most abundant species, but still was present in low numbers (<.5/ ml), and was present on four out of the five macrophyte species.

From my graphic analysis, several conclusions were made about the patterns of toxic dinoflagellates that were also statistically significant. The data was analyzed using a One-way ANOVA and a General linear model with Tukey's comparison. By using the

ANOVA, a correlation between the total species density and individual species relative to the season (Fall-Spring), site (Mahai'ula, Richardson's, Onekahakaha), side (leeward-windward), and seaweed species.

- *G. toxicus* was found on the leeward side of the Big Island in both seasons, showing a distinct preference. *G. toxicus* population density on the leeward side was significantly different from the population density of the windward side ($p=.000$).
- *Prorocentrum* species were found on the windward side of the Big Island in the spring season with the exception of Richardson's. Statistically *Prorocentrum* spp. showed a preference for *Galaxaura marginata* and *Trichleocarpa fragilis* resulting in a p-value of .000.
- *O. lenticularis* was found on both sides of the island and at every sight during both seasons, but had no significance.
- *O. siamensis* was found on both sides of the island and at every site during both seasons, but had no significance.
- *O. ovata* was found only in the fall season. *O. ovata* population density in the fall season was significantly different from the spring season ($p=.010$).
- The total dinoflagellate population had a preference for *Galaxuara marginata* resulting in a p-value of .000.

The General linear model with Tukey's comparison is used to compare each of the above factors within and against each other. The total dinoflagellate and individual dinoflagellate density of each site was compared with each other site, each side to the other and each season to the other. The results showed a significance for only *G. toxicus* and *O. ovata* and are as follows:

- *G. toxicus* at Mahai'ula spring was significantly different from Richardson's spring ($p=.002$), Onekahakaha spring ($p=.001$), Onekahakaha fall ($p=.001$), and Richardson's fall ($p=.002$). Mahai'ula fall was significantly different from every sample group except Mahai'ula spring samples.

- *O. ovata* at Richardson's fall was significantly different from Onekahakaha fall ($p=.015$), Richardson's spring ($p=.004$), and Onekahakaha spring ($p=.003$).

From these results we can determine that *G. toxicus* is predominant on the leeward side of the island and does not have a seasonal variability. *O. ovata* is distinctly a seasonal dinoflagellate, which occurs in the fall season, but only at the Richardson's site. The preference for the two red seaweed's by *Prorocentrum* spp., shows a significant preference for these two species or the group itself. The total dinoflagellate population's preference for *Galaxuara marginata* was severely influenced by the number of *Prorocentrum* spp., but still shows a link to this species.

Using the samples and their replicates, the percent error of the population densities was found. The samples had a 93% error rate, meaning that all samples could be off by nearly a factor of 2. The statistical comparisons that were obtained with this error factor are impressive, showing that even more conclusion could be reached if the error rate was lowered significantly.

Discussion

In conclusion to my first hypothesis, I found that *Gambierdiscus toxicus* was the only species that the population density changed between sites. *G. toxicus* has sighted as the primary causative agent of CFP in French Polynesia, (Chinain 1999a, Chinain 1999b), and in the Caribbean (Lewis et al. 1998), but no study has studied this link in Hawaiian waters. The fact that the seasonal

population difference between fall and spring at Mahai'ula, concurs with a studies done by Ballentine et al. (1985) and Hokama et al. (1996). In another study in Tahiti, *G. toxicus* reached its highest abundance at the end of the hot season, which would explain the slight difference in population density from the fall to the spring (Chinain₂ 1999). Taylor (1985) provides another reason in that *G. toxicus* populations are seriously reduced by high runoff, which is at the time of my second sampling period. This also accounts for the presence of *Ostreopsis* spp. and *Prorocentrum* spp. on the windward, since high runoff does not have an adverse affect on these species (Grzebyk 1993). The results for my second hypothesis showed an overall preference for *Galaxuara marginata* by toxic dinoflagellates. The *Prorocentrum* species showed the same preference for *Galaxuara marginata* and for *Trichleocarpa fragilis*. Previous studies showed population densities to be highest on rhodophytes and phaeophytes, relative to their counterparts, chlorophyta and angiosperms (Morton 1997; Ballantine et al., 1988; Carlson 1984; Bomber 1985). My study showed the same correlation between the density of toxic dinoflagellates and their host being either reds or brown, but this was nullified by the fact that red seaweed's (18 samples), and brown seaweed's (15 samples) made up 86% of the total samples collected. This data agrees with Morton (1997) and Legrand (1999) statement saying that this group of seaweeds may release certain nutrients, vitamins and polyphenols that stimulate the growth of the epiphytic dinoflagellates.

No other seaweed was found to be significantly preferred by dinoflagellates, but *O. ovata* was found repeatedly on rhodophytes in both

seasons. This point is backed by Gryzebyk (1993) in which red macroalgae was found to significantly promote the growth of *O. ovata* and at the same time slowed the growth of *G. toxicus*. This factor accounts again for *G. toxicus*' lower number in the spring, but the fact that the lower number of red seaweed samples in the spring would also explain the decrease in the lower *O. ovata* densities.

In a study done by Morton (1995), data showed that *Prorocentrum lima* from the South and Southeast coasts produced almost three times as much okadaic acid as north and west coasts. Using this data and knowing the extremely high population density of *Prorocentrum* spp. at Richardson's, it would be interesting to look at the tumor growth rate and number on sea turtles around this shoreline.

Conclusions

Although studies have been conducted, the exact factors that control the growth of dinoflagellates are still unknown. My study had high rates of variability, which were caused by the selection of different seaweed's at each site and numerous other factor. The significant results are very pertinent though and show some very distinct patterns that can be used in future studies. *Prorocentrum* species high density can be linked to various nutrients in the macrophytes it was found attached to, or to the water medium that it thrives in. *Gambierdiscus toxicus* is prolific on the West Coast due to the lack of runoff and several other factors. *Ostreopsis ovata* shows a seasonal difference that can be linked to nutrients in the water or a possible lack of suitable habitat in the spring months.

Acknowledgments

I would like to thank Dr. Mike Parsons for making this project possible. I have grown and learned a lot from and am forever grateful. Thank you also to Karla Mcdermid, Leon Hallacher, John Coney and the rest of the UHH Faculty. I would like to especially thank Lynsdey Rock for her support, helpfulness, and her wheels.

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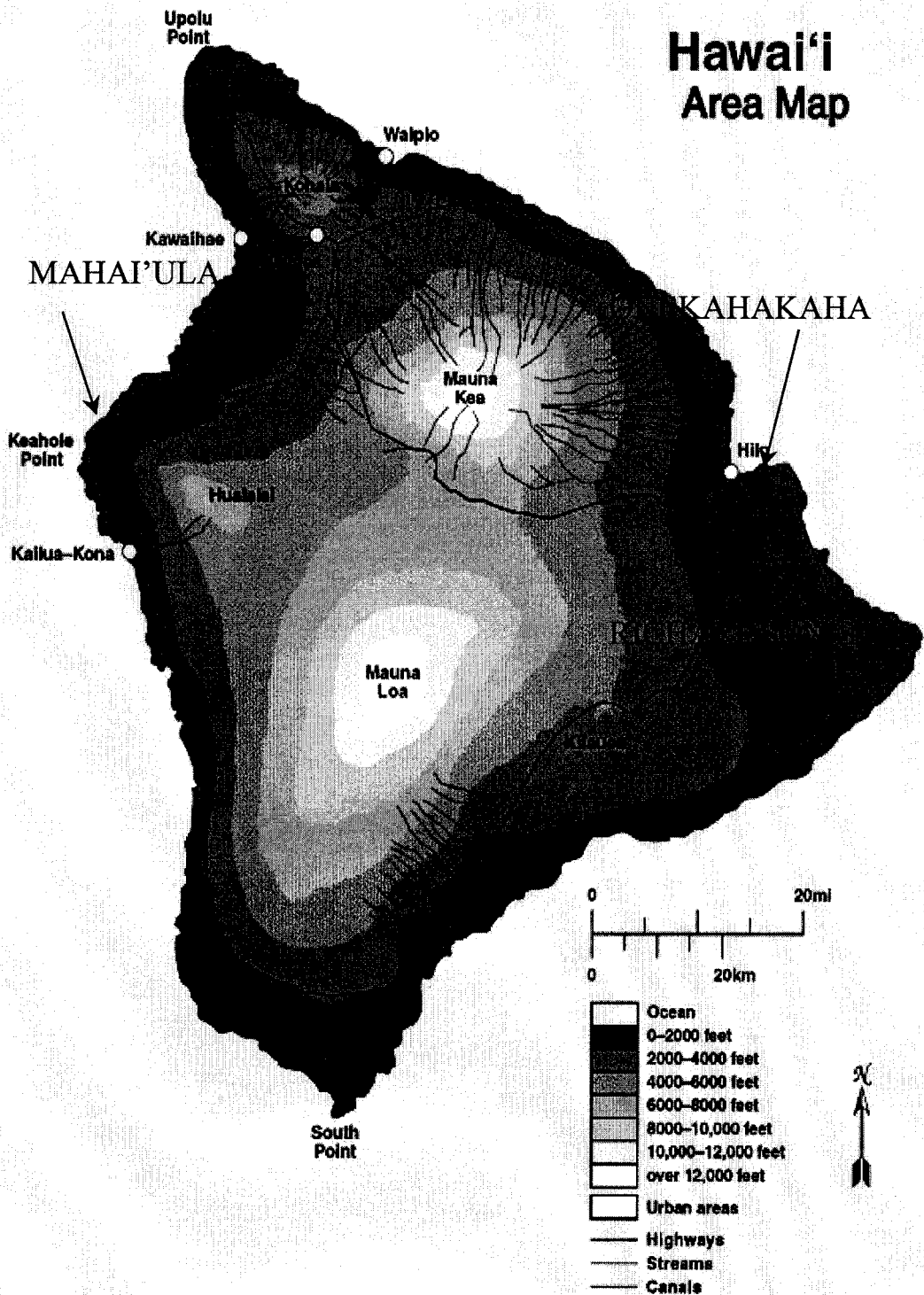
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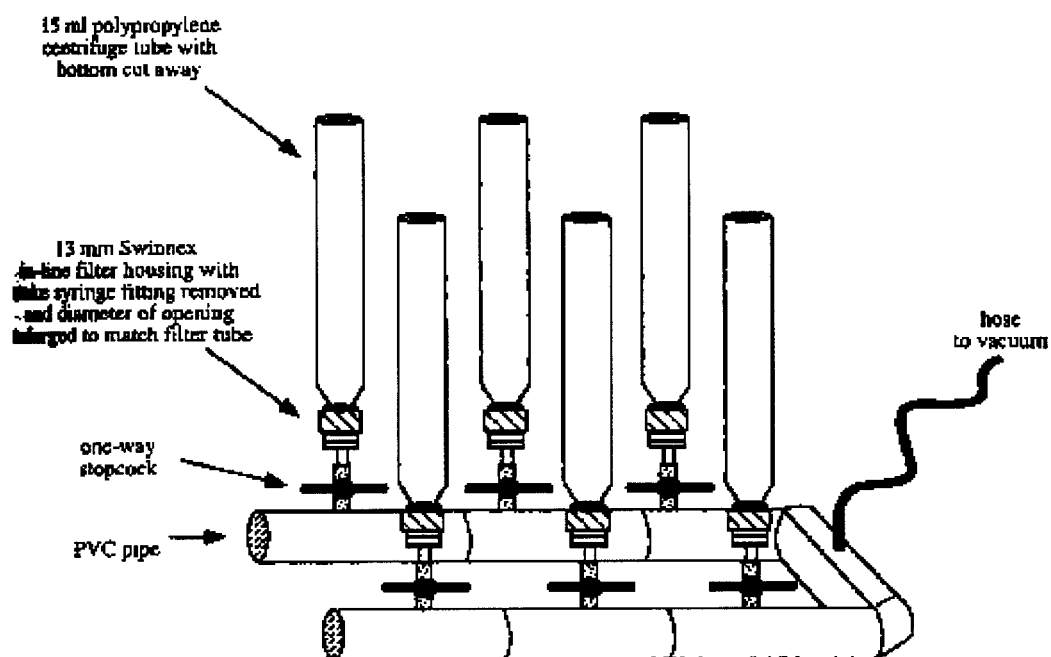
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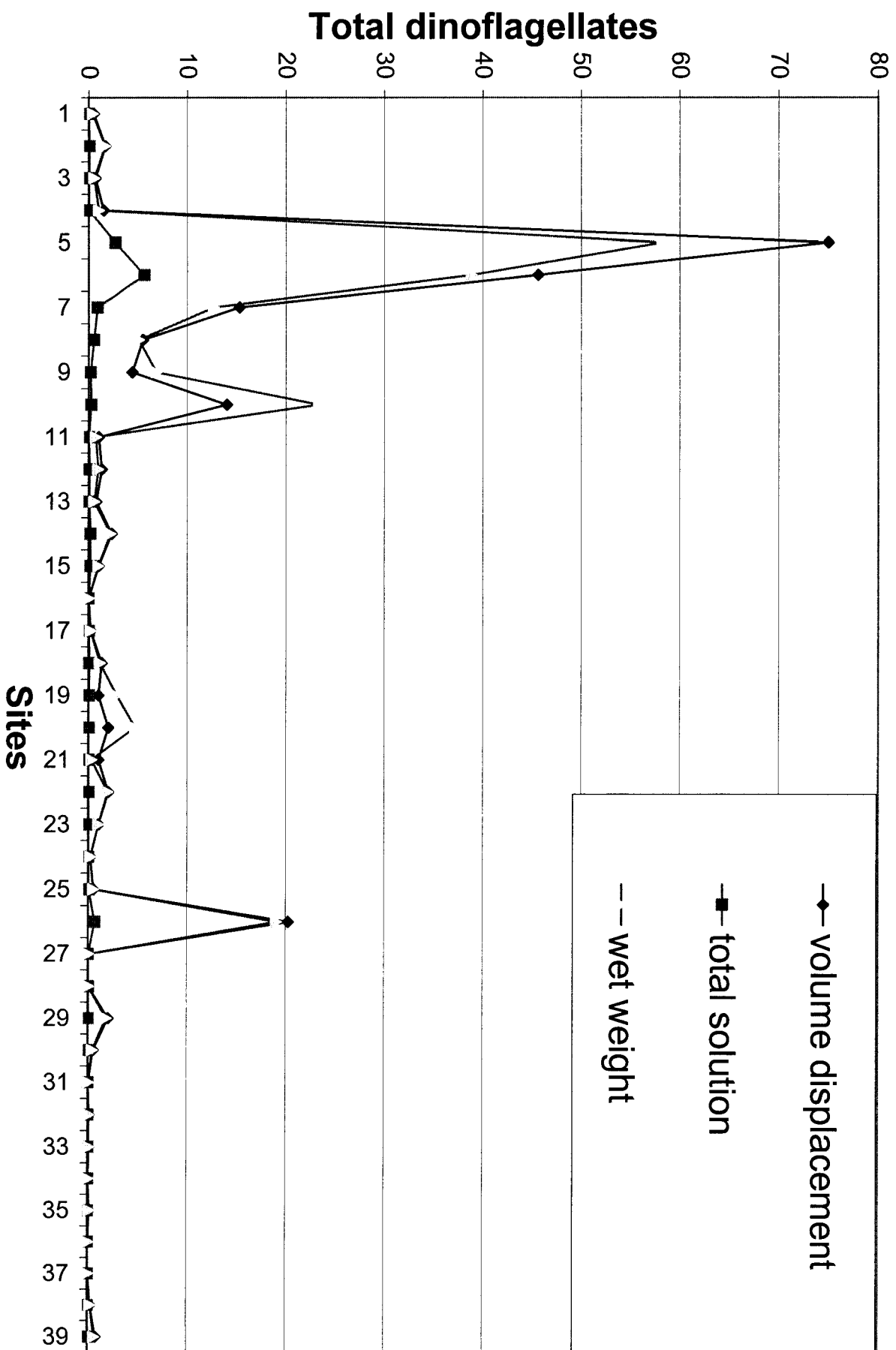
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Figures

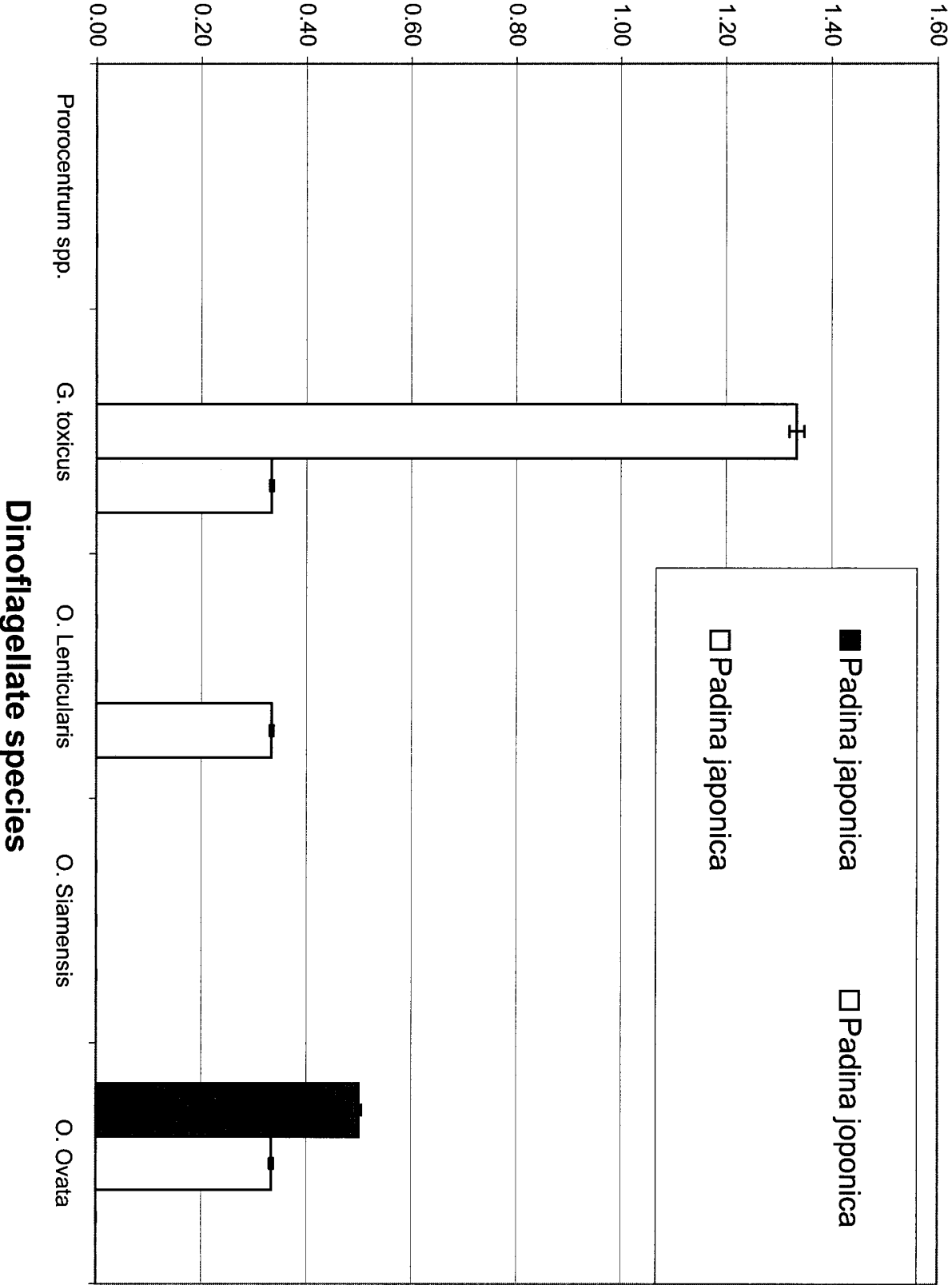
- Fig. 1. General map of the Big Island of Hawai'i. Three sampling sites are shown in bold print signified with arrows.
- Fig. 2. Schematic showing the configuration of a custom filtration manifold design to facilitate filter-based whole-cell hybridization.
- Fig. 3. Comparative values for number of toxic dinoflagellates per milliliter of volume displacement, per milliliter of total solution, per milligram of wet weight. Seaweed's 1-20 are fall samples, seaweed's 21-39 are spring samples. Total dinoflagellates are the total number counted on each seaweed.
- Fig. 4. Mahai'ula fall sample. Each *Padina japonica* was collected a different location. *G. toxicus* was present in the highest numbers, *O. lenticularis*, and *O. ovata* were also present.
- Fig. 5. Richardson's fall sample. All toxic species showed up at this site with *Prorocentrum* spp. being the especially dense, and *O. ovata* being second highest.
- Fig. 6. Onekahakaha fall sample. *O. ovata* was the most abundant and was present on all seaweed's. *O. siamensis* and *O. lenticularis* were also present.
- Fig. 7. Mahai'ula spring sample. *O. lenticularis* was the most abundant. *G. toxicus* was also present on two of the samples. Each seaweed only held one dinoflagellate species. Each sample was from a different location.
- Fig. 8. Richardson's spring sample. All toxic species showed up at this site with *Prorocentrum* spp. being the most abundant. All other densities were very low.
- Fig. 9. Onekahakaha spring sample. *Prorocentrum* spp. was the most abundant species. *O. lenticularis* was the only other species present.

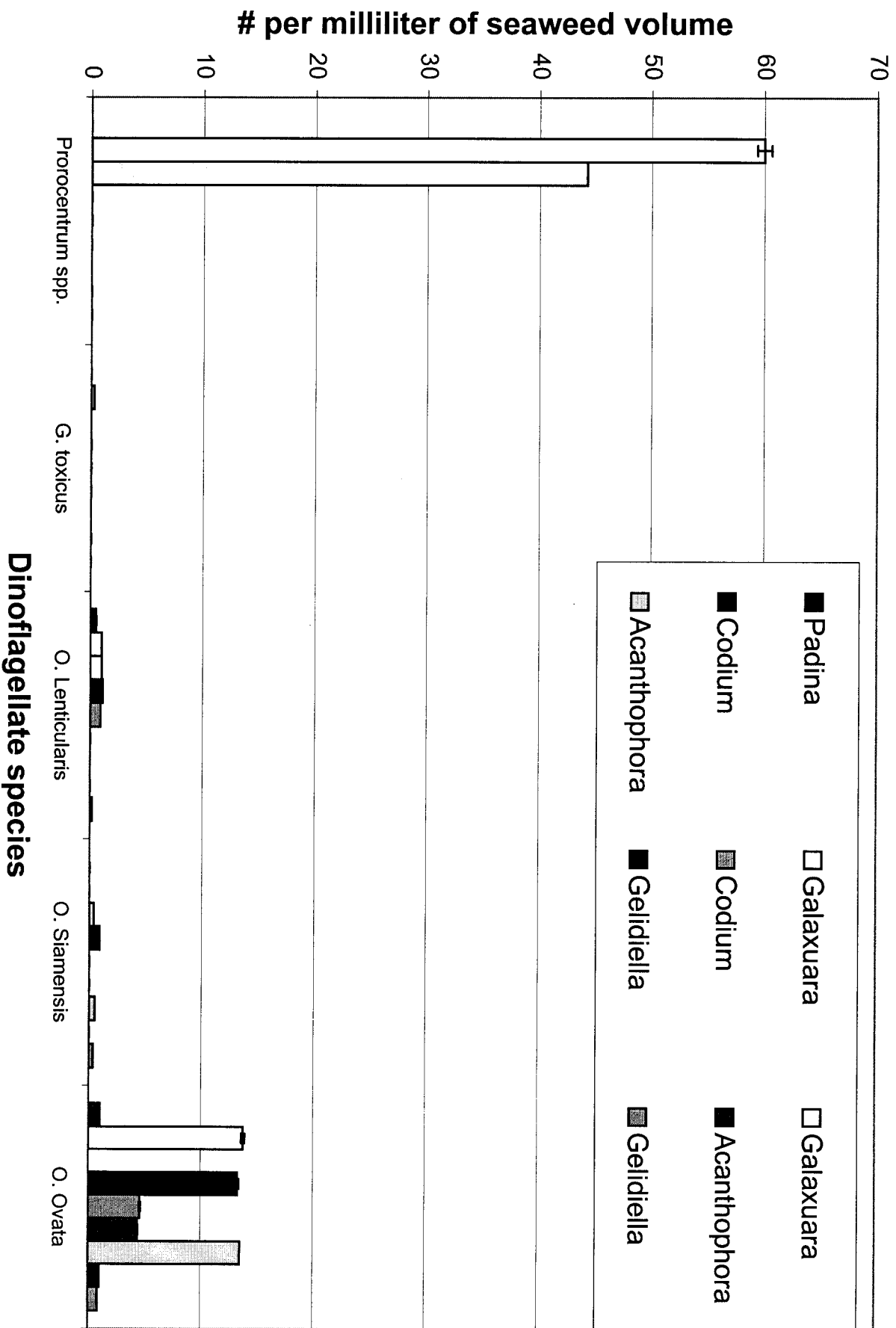


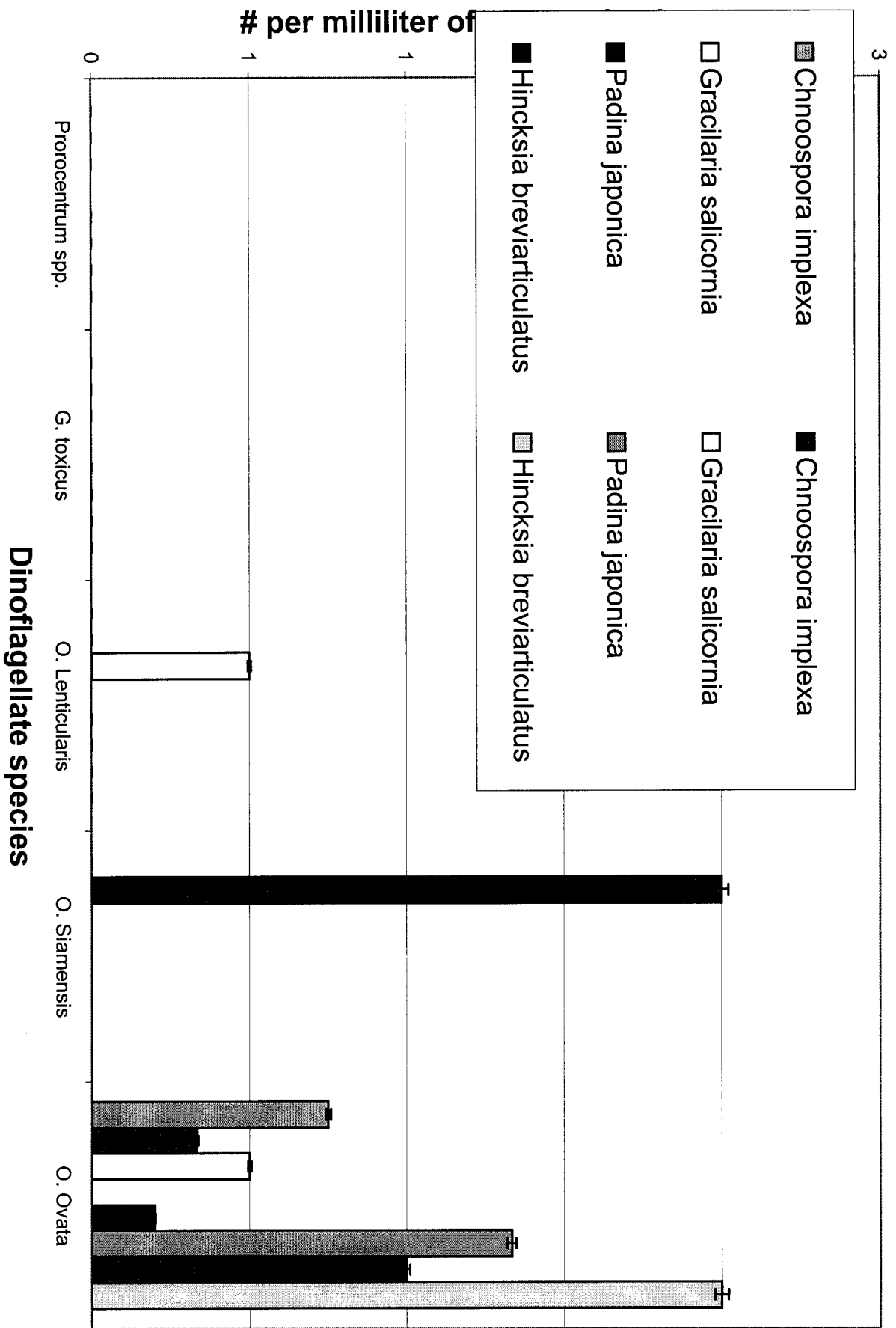


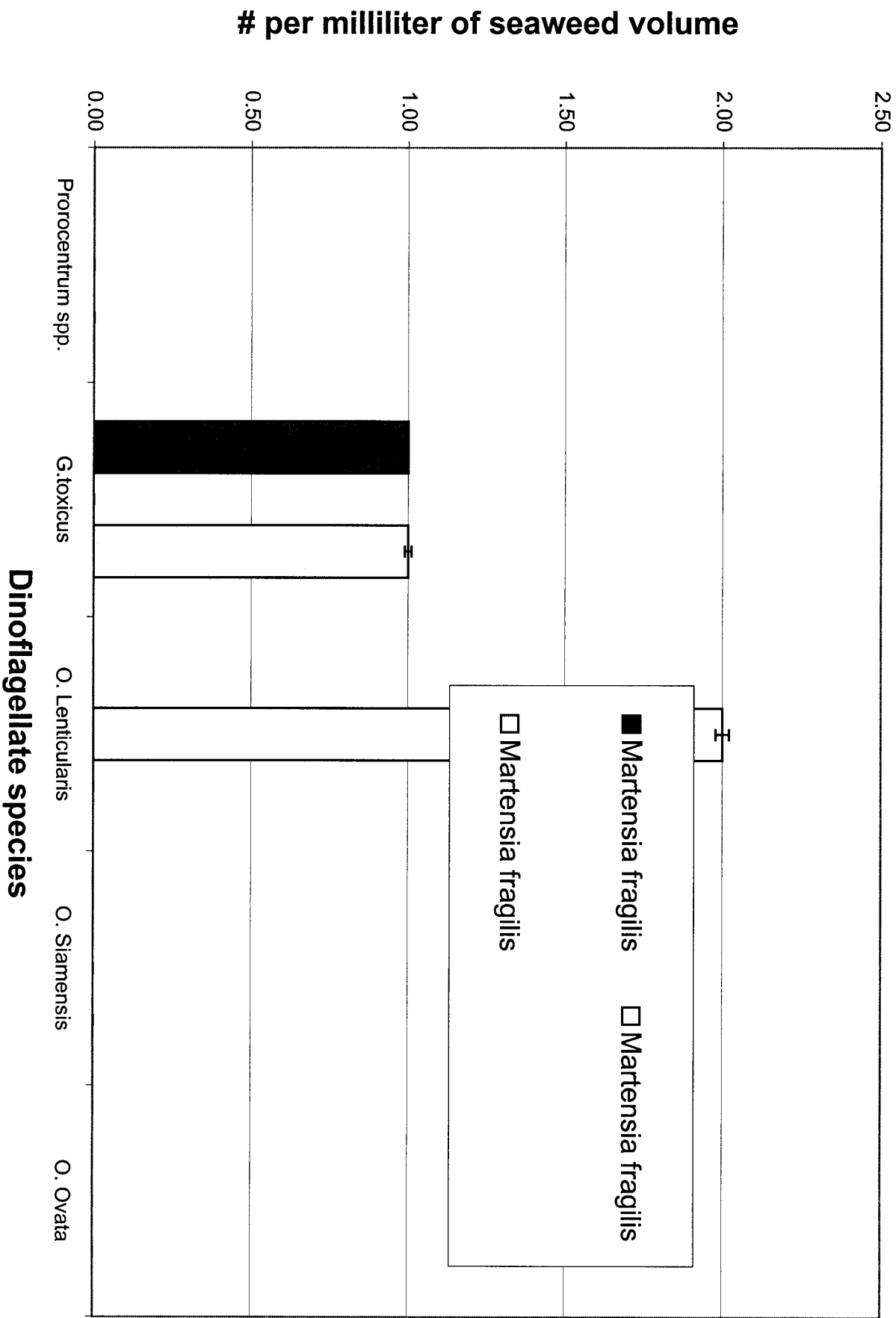


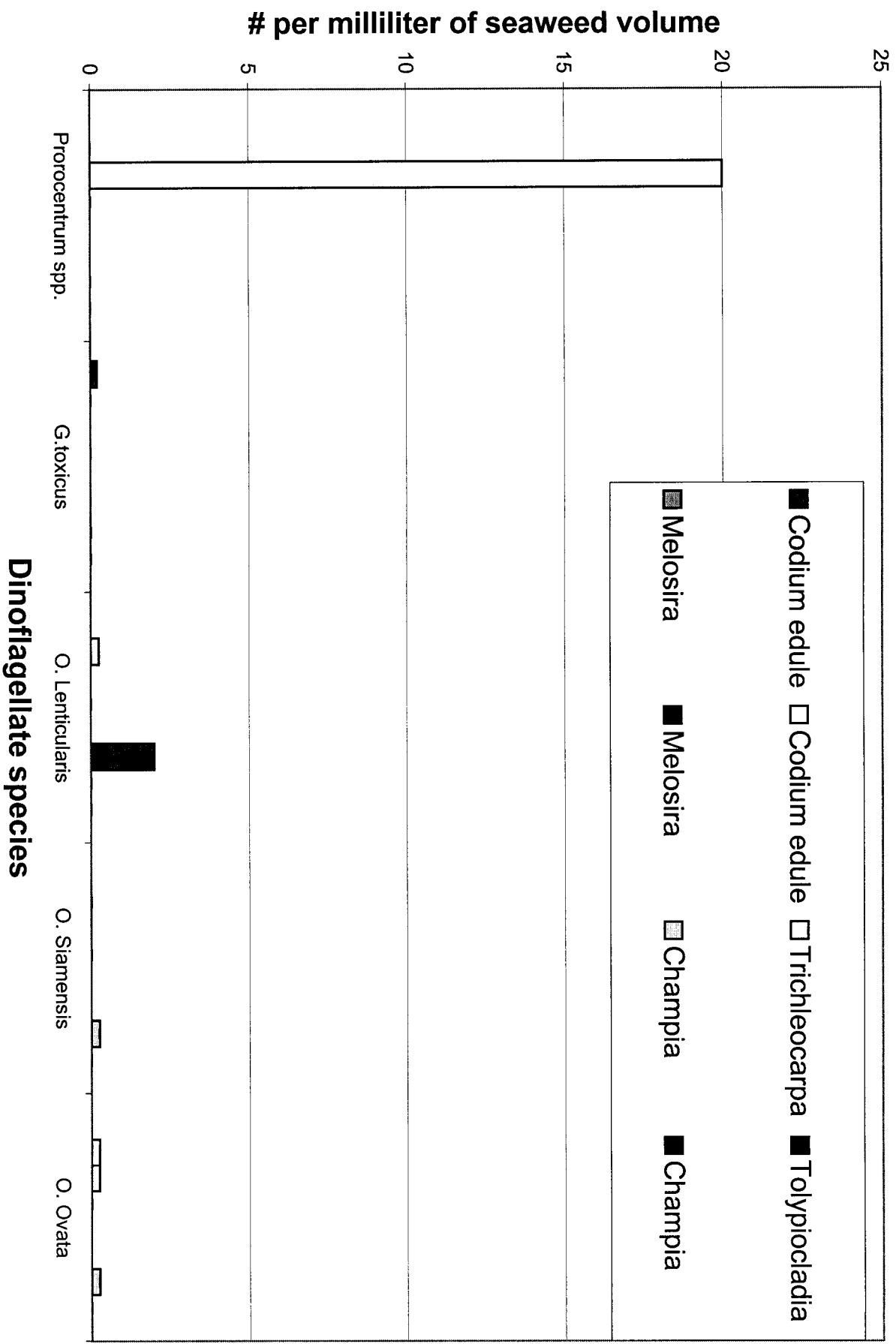
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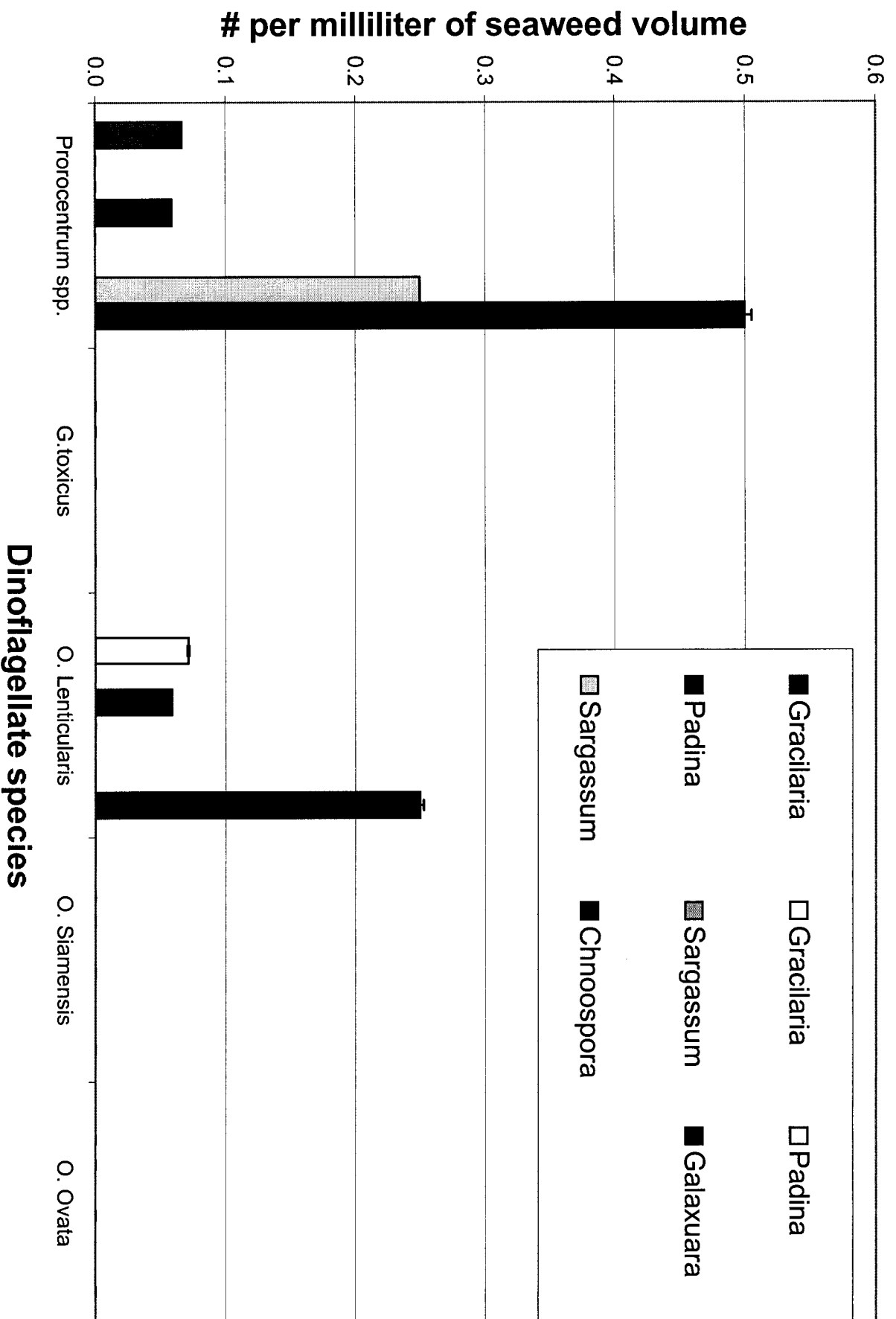












THE MOP PROGRAM AND WHAT I BENEFITED

As a freshman in my first year at the University of Hawaii at Hilo, I had no idea of what the future held for me. The biggest plans I had were to play baseball and complete my class schedule for the year. When I first took a tour of the campus, they stopped by a little building painted with a big wave on the side, upon which they told me this was where the marine science students hung out and did a lot of their work. I stepped in and was very intimidated with what I saw.

As the year went on I had baseball practice and games which did not allow for any of the great looking MOP trips. Every time I stopped into the mop office I saw the same people always doing their work and hanging out and thought these people were crazy. I never really came back to the mop shop until my senior year and now I don't understand how I went without this building and this program.

From getting a job on the Four Winds boat to working on my senior thesis, I could honestly say I spent my senior year living in the MOP office and can understand why I always saw the same people in here all the time. Since the beginning of the year I have made numerous friends and met faculty members from different schools. Being around this environment has taught me how to conduct myself in a professional manner, while still enjoying myself. While learning the huge benefit of networking with people that actually know something, I have learned what it is like to enter the real world and be prepared for what is to come.

After completing my senior project it has occurred to me that I set out with a goal and a deadline to be completed by and realized that I can actually do this. The most important thing I learned though was how to conduct research that is worthy of the scientific society. I had no idea about the amount of time and effort, or even the basics of building a study. Now I feel I am ready to take the next step and become a scientist, which is what the MOP program and Marine Science department are there to do. I do regret not being able to attend the MOP trips, but the valuable experiences I have learned as a person and as a professional could not have been learned anywhere else. For this I am very thankful to John Coney and the MOP program, and the Marine Science faculty who showed me the personal attention that only comes from small school.

Thank you very much to everybody,

Ian C. Robbins